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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR7431 for a patent by INTREAT PTY LIMITED as filed on 03 September 2001.

I further certify that pursuant to the provisions of Section 38(1) of the Patents Act 1990 a complete specification was filed on 17 January 2002 and it is an associated application to Provisional Application No. PR7431 and has been allocated No. 2002224664.

WITNESS my hand this
Twelfth day of March 2007

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES

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PROVISIONAL SPECIFICATION
FOR A PROVISIONAL PATENT

Name of Applicant: **INTREAT PTY LIMITED**
Actual Inventor: **JULIAN ALEXANDER BARDEN & ANGUS GIDLEY-BAIRD**
Address for Service:

Chrysiliou Law
CMC Centre
143 Sydney Road
Fairlight
Sydney NSW 2094

Invention Title: **A Cancer Therapeutic**

The following statement is a description of this invention

This invention relates to antibody-based treatments of cancers, especially those derived from epithelial cells and malignant lymphoma.

Part of the basis for the invention is found in research into the purinergic receptor $P2X_7$ in epithelial cells. The $P2X_7$ subtype is involved in apoptosis or programmed cell death in many cell types including epithelial cells. In cancer cell lines, such as human prostate PC3 and human breast MCF7, the $P2X_7$ receptor is found on the cell surface but all in a non-functional conformation. Normal $P2X_7$ is a cytolytic receptor capable of forming pores that enable the cell to be flooded with excess calcium rather than simply acting as a calcium channel. Adenosine triphosphate (ATP) can induce cytolysis in cells such as leukocytes including lymphocytes, thymocytes, macrophages and dendritic cells through the $P2X_7$ receptors expressed on the cell surface. $P2X_7$ receptors open channels through the cell membrane within a second. Continued application of ATP leads to the formation of a pore within a few seconds to tens of seconds that induces apoptosis. This process is not completed in the epithelial cancer cells.

Prior patent application No. PR 5890, the contents of which are imported herein by reference, refers to a $P2X_7$ subtype-specific antibody that specifically binds to non-functional $P2X_7$ receptors expressed on epithelial cells forming part of preneoplastic or neoplastic tissue and on any neoplastic cell expressing non-functional $P2X_7$ receptors. In patients with epithelial cell cancer such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder and vaginal cancer, and malignant lymphoma, but not confined to these, the non-functional $P2X_7$ receptors can be detected by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors so that the cause of the conformational change in the receptors relates to interaction of the receptors with ATP. The ATP molecules act as receptor agonists, so that when ATP is bound to the receptors, they are able to open a channel through the cell membrane for the flow of calcium ions. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors. If ATP binding to the receptors is disrupted, the receptor conformation is altered and this can be detected using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP.

The specific sequence involved in the conformational change includes Pro210 in human $P2X_7$ receptors that undergoes a change in configuration from trans form to cis form in the absence of bound ATP. Thus an appropriate epitope sequence against which an antibody must be raised includes Pro210 and may extend either side of this residue to an

appropriate extent necessary to induce an antibody response. This may include by way of example a segment extending from Gly200-Thr215 but is not confined to this segment.

Because current studies and investigations may not fully explain the working of the invention, it is necessary to define the invention in a number of aspects, as set out below. It is possible and likely that there will be overlap of at least some of those aspects.

Accordingly, in a first aspect, the invention provides an epitope capable of causing the generation of an antibody for binding to cells in infected, cancer and/or preneoplastic tissue, including those cancers derived in epithelial cells listed above, the antibody being adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors. Tissue may be infected by agents such as a virus, for example HIV, or other pathological organisms that may have caused non-functional receptors to be expressed, either directly through inhibition of co-factors required for functionality or through the up-regulation of co-factors acting to inhibit $P2X_7$ function on epithelial or other cells so rendering the infected cell less amenable to destruction by the immune system.

The epitope preferably includes Pro210 in the human $P2X_7$ sequence and encompassing the segment Gly200-Thr215. This epitope should preferably have attached to the C-terminal end a Cys residue that is cross-linked to diphtheria toxin via the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS) so that the conformation adopted by the attached epitope peptide occupies a stable cis proline configuration.

This specific peptide conformation is intended to be presented to humans with one or more different epithelial cell cancers or patients that may have been infected with agents such as a virus, for example HIV, or other pathological organisms that may have caused non-functional receptors to be expressed, either directly through inhibition of co-factors required for functionality or through the up-regulation of co-factors acting to inhibit $P2X_7$ function on epithelial or other cells so rendering the infected cell less amenable to destruction by the immune system. The patients will preferably mount an immune response to the applied conjugated epitope so that an antibody or immune response will be mounted by the affected patients and so generate antibodies recognising the non-functional $P2X_7$ receptors that are present on the surface of the affected cancer cells or other cells affected by pathological agent, thus binding to them and alerting the appropriate immune cells to destroy the complexed cancer or other cells. Such other cells may be erythrocytes or other cells primed for cell death.

It is apparent that the invention covers alternative sequences and carriers and cross-linkers that similarly produce a specific immune response against preferably only non-functional $P2X_7$ receptors, preferably ignoring all functional receptors expressed on cell surfaces and so avoiding side effects.

In a second aspect, the invention provides for the use of the epitope of the invention as a therapeutic vehicle for treatment of infection or cancer in a patient to regulate programmed cell death by causing the generation of an antibody to target the aberrant or non-functional $P2X_7$ receptors expressed on the surface of the cells while leaving all cells expressing normal receptors untouched.

In a third aspect, the invention provides a pharmaceutical composition for treatment or prevention of disease or medical conditions in a patient, including cancers in the patient, the composition including a pharmaceutically effective amount of an epitope capable of causing the generation of an antibody able to regulate programmed cell death of cells, these cells having expressed on their surface aberrant or non-functional $P2X_7$ receptors.

The invention also provides a method of treating or preventing cancer or medical diseases in a patient, comprising administering to the patient a pharmaceutical composition as defined above.

The invention also provides the use of a pharmaceutical composition defined as above in the treatment or prevention of disease or medical conditions, including epithelial derived cancers and malignant lymphoma, in a patient.

In a further aspect, the invention provides a method for distinguishing between different conformations of proteins by using an epitope capable of causing the generation of an antibody, or the antibody itself, to effect specific pharmaceutical outcomes from binding to all members of the proteins with a selected conformation. An example of this would be prion proteins in the conformation that leads to the condition vCJD. The abnormal form of the protein could be targeted by a specific antibody or epitope causing the generation of the antibody, preferably humanised and reduced in size for optimum pharmacological effect.

It will be apparent to those skilled in the art that many obvious modifications and

variations may be made to the embodiments described herein without departing from the spirit or the scope of the invention.

Dated this 3rd day of September, 2001

Intreat Pty Limited

by its Patent Attorneys

Chrysiliou Law